Amendments to the Claims

A detailed listing of all claims in the application is presented below. This listing of claims replaces all prior versions of the claims in the application. All claims currently amended are submitted with markings to indicate the changes relative to the immediate prior version of the claims. The changes in any amended claim are shown by strikethrough (for deleted matter) or underlined (for added matter).

1. (Original) A recombinant DNA comprising said DNA selected from the group consisting of:

   a) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

   b) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

   c) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

   d) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

   e) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

   f) any portion of said DNA above that encodes a protein that elicits an immune response against E. canis.

2. (Original) The recombinant DNA of claim 1 wherein said DNA encodes at least one immunogenic epitope.

3. (Original) A recombinant protein comprising said protein selected from the group consisting of:

   a) a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

b) a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

c) a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

d) a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

e) a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

f) any portion of any of the above proteins that elicits an immune response against *E. canis*.

4. (Original) The recombinant protein of claim 3 wherein said protein includes at least one immunogenic epitope.

5. (Original) A vaccine wherein said vaccine protects dogs against *E. canis* infection.

6. (Original) A vaccine comprising:

a) a vector capable of expressing a recombinant DNA inserted into said vector such that a recombinant protein is expressed when said vector is provided in an appropriate host; and

b) the recombinant DNA inserted into said vector wherein said DNA is selected from the group consisting of:

i) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi) any portion of said DNA above that encodes a protein fragment that is greater than 25 amino acids.

7. (Original) The vaccine of claim 6, wherein said DNA further comprises DNA that encodes CpG motifs.

8. (Original) The vaccine of claim 6 wherein said DNA further comprises a promoter selected from the group consisting of:

a) a cytomegalovirus (CMV) immediate early promoter;

b) a human tissue plasminogen activator gene (t-PA); and

c) promoter/enhancer region of a human elongation factor alpha (EF-1 α).

9. (Original) The vaccine of claim 6, wherein said vector is selected from the group consisting of:

a) pcDNA3;

b) pC1;

c) VR1012; and

d) VR1020.

10. (Original) The vaccine of claim 6 wherein said vaccine is administered into said host by a method selected from the group consisting of:

a) intramuscular injection;

b) intravenous injection; and

c) gene gun injection.

11. (Original) The vaccine of claim 10, wherein said host is a dog.

12. (Original) The vaccine of claim 5 comprising:

a) a recombinant protein that is selected from the group consisting of:

i) a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii) a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii) a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv) a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v) a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi) any portion of any of the above proteins that elicits an immune response against *E. canis*.

13. (Original) The vaccine of claim 12, wherein said vaccine further comprises adjuvants selected from the group consisting of:

a) aluminum hydroxide;

b) QuilA; and

c) Montamide.

14. (Original) The vaccine of claim 12 further comprising a cytokine operatively associated with said recombinant protein.

15. (Original) The vaccine of claim 14 wherein said cytokine is selected from the group consisting of:

a) interleukin-1β (IL-1β);

b) granulocyte-macrophage colony stimulating factor (GM-CSF);

c) gamma interferon (γ-IFN);

d) amino acids VQGEESNDK from the IL-Iβ protein; and

e) any portion of any of the cytokines above that elicits an improved immunogenic response against *E. canis*.

16. (Original) The vaccine of claim 12 wherein said vaccine is administered into a host by a method selected from the group consisting of:

a) intramuscular injection; and

b) subcutaneous injection.

17. (Original) The vaccine of claim 16 wherein said host is a dog.

18. (Original) The vaccine of claim 5 comprising a recombinant protein that includes a T cell epitope wherein said T cell epitope comprises an amino acid peptide fragment of a protein selected from the group consisting of:

a) a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

b) a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

c) a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

d) a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

e) a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

f) any portion of any of the above proteins that elicits an immune response against *E. canis*.

19. (Original) The vaccine of claim 18 wherein said amino acid peptide fragment comprises nine to twenty amino acids.

20. (Original) The vaccine of claim 18 further comprising a recombinant DNA encoding a protein which is capable of being internalized into eukaryotic cells, including cells of the immune system.

21. (Original) The vaccine of claim 20 wherein said protein capable of being internalized into eukaryotic cells comprises a toxin selected from the group consisting of:

a) a recombinant adenylate cyclase of *Bordetella bronchiseptica*; and

b) a recombinant exotoxin A (PE) of *Pseudomonas aeruginosa*.

22. (Original) The vaccine of claim 18 wherein said vaccine is administered into a host by a method selected from the group consisting of:

a) intramuscular injection; and

b) subcutaneous injection.

23. (Original) The vaccine of claim 22 wherein said host is a dog.

24. (Currently amended) A method of identifying a T cell epitope against *E. canis* comprising:

a) synthesizing overlapping peptide fragments over an entire length of a protein of claim 3, ~~wherein said protein is selected from the group consisting of:~~

~~i) a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;~~

~~ii) a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;~~

~~iii) a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;~~

~~iv) a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;~~

~~v) a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and~~

~~vi) any portion of any of the proteins above that elicits an immune response against *E. canis*;~~

b) testing said peptide fragment to determine if said peptide fragment elicits an immune response in a host animal; and

c) identifying said peptide fragment as said T cell epitope of *E. canis* if said fragment elicits an immune response.

25. (Original) The method of claim 24 wherein said peptide fragment comprises nine to twenty amino acids.

26. (Original) A method of creating a vaccine against *Ehrlichia canis* comprising:

a) selecting a vector capable of expressing a recombinant DNA inserted into said vector; and

b) inserting a recombinant DNA into said vector such that a recombinant protein is expressed when said vector is provided in an appropriate host wherein said DNA is selected from the group consisting of:

i) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi) any portion of said DNA above that encodes a protein fragment that is greater than 25 amino acids.

27. (Original) The method of claim 26, wherein said DNA further comprises DNA that encodes CpG motifs.

28. (Original) The method of claim 26 wherein said DNA further comprises a promoter selected from the group consisting of:

a) a cytomegalovirus (CMV) immediate early promoter;

b) a human tissue plasminogen activator gene (t-PA); and

c) a promoter/enhancer region of a human elongation factor alpha (EF-1 α).

29. (Original) The method of claim 26, wherein said vector is selected from the group consisting of:

a) pcDNA3;

b) pC1;

c) VR1012; and

d) VR1020.

30. (Original) The method of claim 26 wherein said vaccine is injected into said host in a manner selected from the group consisting of:

a) intramuscular injection;

b) intravenous injection; and

c) gene gun injection.

31. (Original) The method of claim 30, wherein said host is a dog.

32. (Original) A method of creating a vaccine against *E. canis* comprising:

a) selecting a vector capable of expressing a recombinant protein inserted into said vector;

b) insertion of a recombinant DNA into said vector such that said recombinant protein is expressed when said vector is transformed into a bacterial strain wherein said DNA is selected from the group consisting of:

i) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi) any portion of said DNA above that encodes a protein that elicits an immune response against *E. canis*; and

c) harvesting said recombinant protein from said bacterial strain.

33. (Original) The method of claim 32, wherein said vaccine further comprises adjuvants selected from the group consisting of:

a) aluminum hydroxide;

b) QuilA; and

c) Montamide.

34. (Original) The method of claim 32, wherein said vaccine further comprises a promoter selected from the group consisting of:

a) tac;

b) T5; and

c) T7.

35. (Original) The method of claim 32, wherein said bacterial strain is *E. coli*.

36. (Original) The method of claim 32, wherein said vector is selected from the group consisting of:

a) pREST;

b) pET; and

c) pKK233-3.

37. (Original) The method of claim 32 wherein said vaccine further comprises a cytokine operatively associated with said vaccine.

38. (Original) The method of claim 37 wherein said cytokine is selected from the group consisting of:

a) interleukin-1β (IL-1β);

b) granulocyte-macrophage colony stimulating factor (GM-CSF);

c) gamma interferon (γ-IFN);

d) amino acids VQGEESNDK from the IL-Iβ protein; and

e) any portion of any of the cytokines above that elicits an improved immunogenic response against *E. canis*.

39. (Original) The method of claim 32 wherein said vaccine is injected into said host in a manner selected from the group consisting of:

a) intramuscular injection; and

b) subcutaneous injection.

40. (Original) The method of claim 39 wherein said host is a dog.

41. (Currently amended) A method of creating a T cell epitope vaccine comprising:

a) selecting a recombinant protein that includes a T cell epitope wherein said T cell epitope comprises an amino acid peptide fragment of a protein of claim 3, ~~selected from the group consisting of:~~

~~i) a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;~~

~~ii) a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;~~

~~iii) a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;~~

~~iv) a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;~~

~~v) a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and~~

~~vi) any portion of any of the above proteins that elicits an immune response against *E. canis*;~~

b) identifying said T cell epitope from said protein;

c) incorporating said T cell epitope into a construct capable of expressing said epitope as a protein; and

d) harvesting said protein.

42. (Original) The method of claim 41 wherein said amino acid peptide fragment comprises nine to twenty amino acids.

43. (Original) The method of claim 41 wherein said construct capable of expressing said epitope further comprises a recombinant DNA encoding a protein which is capable of being internalized into eukaryotic cells, including cells of the immune system.

44. (Original) The method of claim 43 wherein said protein capable of being internalized into eukaryotic cells comprises a toxin selected from the group consisting of:

a) a recombinant adenylate cyclase of *Bordetella bronchiseptica*; and

b) a recombinant exotoxin A (PE) of *Pseudomonas aeruginosa*.

45. (Original) The method of claim 41 wherein said vaccine is injected into said host in a manner selected from the group consisting of:

a) intramuscular injection; and

b) subcutaneous injection.

46. (Original) The method of claim 45 wherein said host is a dog.

47. (Original) A recombinant DNA comprising said DNA selected from the group consisting of

a) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

b) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

c) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ.ID. NO. 7;

d) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9; and

e) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11.

48. (Original) A vector capable of expressing a recombinant DNA comprising:

a) a recombinant DNA inserted into said vector such that a recombinant protein is expressed when said vector is provided in an appropriate host wherein said DNA is selected from the group consisting of:

i) a recombinant DNA sequence that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii) a recombinant DNA sequence that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii) a recombinant DNA sequence that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv) a recombinant DNA sequence that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi) any portion of said DNA above that encodes a protein that elicits an immune response against *E. canis*.

49. (Original) The recombinant DNA of claim 47 wherein said DNA encodes at least one immunogenic epitope.

50. (Original) A vector capable of expressing a recombinant DNA comprising:

a) a recombinant DNA inserted into said vector such that a recombinant protein is expressed when said vector is provided in an appropriate host wherein said DNA is selected from the group consisting of:

i) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9; and

v) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11.

51. (Original) Serological diagnosis techniques using:

a) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

b) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

c) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

d) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9; and

e) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11.

52. (Original) The method of kinetic enzyme-linked immunosorbent assay comprising the steps of:

a) selecting an antigen to be added to microtiter plates that includes an immunogenic epitope comprising a recombinant protein selected from the group consisting of:

i) a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii) a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii) a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv) a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v) a protein having an amino acid sequence as shown in SEQ. ID. NO. 11;

vi) any portion of said DNA above that encodes a protein that elicits an immune response against *E. canis*;

b) adding an antiserum of the species allowing it to complementarily bind to the antigen;

c) adding the antibody to the microtiter plate, allowing the antibody to bind to the antigen;

d) washing the microtiter plate to remove any unbound antibodies;

e) adding an enzyme the microtiter plates allowing the enzyme to bind to the antibody;

f) washing the microtiter plate to remove any unbound enzyme; and

g) adding the enzyme's substrate, allowing it to bind to the enzyme, which produces a color change when bound.

53. (Original) The method of claim 52, where said species is a canine.

54. (Original) The method of claim 52, wherein antiserum added to the microtiter plate is goat anti-canine.

55. (Original) The method of claim 52, wherein the antibody added to the microtiter plate is second antibodies of a goat anti-canine antibody of heavy and light chain specificity.

56. (Original) The method of claim 52, wherein the enzyme added to the microtiter plate is horseradish peroxidase.

57. (Original) The method of claim 52, wherein the enzyme's substrate is chromogen tetramethylbenzidine with H2O2.

58. (Original) The method of western blot analysis comprising the steps of:

a) obtaining the species serum with antigens, where said antigen includes an immunogenic epitope comprising a recombinant protein selected from the group consisting of:;

i) a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii) a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii) a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv) a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v) a protein having an amino acid sequence as shown in SEQ. ID. NO. 11;

vi) any portion of said DNA above that encodes a protein that elicits an immune response against *E. canis*;

b) running the serum through sodium dodecyl sulfate-polyacrylamide gel electrophoresis, allowing proteins to be fractionated into a series of bands arranged in order of molecular weight;

c) transferring the proteins to a filter by blotting;

d) adding antibodies tagged with a dye are washed over the filter, allowing the antibodies to bind to the fractionated proteins; and

e) adding substrates to develop the bands on the filter.

59. (Original) The method of claim 58, wherein said species is a canine.

60. (Original) The method of claim 58, wherein the antibodies are goat anti-dog igG conjugated to horseradish peroxidase.

61. (Original) The method of claim 58, wherein the substrates added to develop the bands on the filter are:

a) 4 chloro-1-napthol in methyl alcohol;

b) tris-buffer solution with a pH of 7.5; and

c) 30% H2O2.

62. (Original) The method of polymerase chain reaction comprising the steps of:

a) selecting a target strand of DNA that will serve as a template for DNA synthesis comprising recombinant DNA selected from the group consisting of:

i) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi) any portion of said DNA above that encodes a protein that elicits an immune response against *E. canis*;

b) adding a mixture containing enzymes, nucleotides, DNA polymerase, and primers;

c) subjecting above mixture to a number of cycles of amplification in an automated DNA cycler; and

d) using products of said cycles of amplification and performing gel electrophoresis.

63. (Original) The method of claim 62, wherein the mixture is comprised of:

a) 50 mM KCl;

b) 10mM Tris-HCl with a pH of 8.3;

c) 1.5 mM MgCl2;

d) 0.5% NP40;

e) 0.5% Tween 20;

f) 200 mM each of deoxynucleoside triphosphates;

g) 2 mM of primer sets; and

h) 2 U of thermostable Taq DNA polymerase.

64. (Original) The method of claim 62, wherein the said number of cycles of amplification is 40.

65. (Original) The method of claim 62, wherein the said cycles of amplification are comprised of:

a) heating to 94°C for 1 minute to allow the DNA to denature;

b) cooling to 69°C for 1 minute to allow the primers to anneal; and

c) heating to 72°C for 2 minutes to allow for primer extension.